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REVIEW

Structure and Epigenetics of Nucleoli in Comparison With Non-nucleolar Compartments

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SUMMARY The nucleolus is a nuclear compartment that plays an important role in ribosome biogenesis. Some structural features and epigenetic patterns are shared between nucleolar and non-nucleolar compartments. For example, the location of transcriptionally active mRNA on extended chromatin loop species is similar to that observed for transcriptionally active ribosomal DNA (rDNA) genes on so-called Christmas tree branches. Similarly, nucleolus organizer region–bearing chromosomes located a distance from the nucleolus extend chromatin fibers into the nucleolar compartment. Specific epigenetic events, such as histone acetylation and methylation and DNA methylation, also regulate transcription of both rRNA- and mRNA-encoding loci. Here, we review the epigenetic mechanisms and structural features that regulate transcription of ribosomal and mRNA genes. We focus on similarities in epigenetic and structural regulation of chromatin in nucleoli and the surrounding non-nucleolar region and discuss the role of proteins, such as heterochromatin protein 1, fibrillarin, nucleolin, and upstream binding factor, in rRNA synthesis and processing.
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EPIGENETIC PROCESSES are heritable changes in the genome that are independent of changes in DNA sequences. DNA methylation and histone modification are the main epigenetic events that regulate nuclear processes such as transcription. Changes in DNA methylation and the histone signature can be observed not only within non-nucleolar genomic regions but also during transcription of ribosomal DNA (rDNA). The nucleolus is the site of ribosome biogenesis and is surrounded by clusters of genes that encode rRNA. During mitosis, rRNA synthesis is turned off due to phosphorylation of specific nucleolar factors. This causes disassembly of nucleoli, which recover at the end of mitosis (Dimario 2004; Leung et al. 2004; Olson and Dundr 2005).

Eukaryotic genomes contain hundreds of head-to-tail tandem repeats called nucleolus organizer regions (NORs). In human diploid cells, which contain ~400 rRNA genes, these regions map to five pairs of acrocentric chromosomes: 13–15, 21, and 22 (Henderson et al. 1972). In the mouse genome, NORs are found on chromosomes 12 and 15–19 (Dev et al. 1977; McStay and Grummt 2008). The position of NORs on the short arms of acrocentric chromosomes isolates them from genes transcribed by RNA polymerase II (Pol II) and Pol III. Nucleolar rRNA genes are transcribed by Pol I, and their expression is regulated by chromatin remodeling factors and some specific epigenetic events (McStay and Grummt 2008). However, the transcription and

silencing of genes encoding rRNAs is governed by histone modification, as occurs with non-nucleolar genes.

rRNA genes provide an interesting model for studying how epigenetic processes and chromatin arrangements can regulate gene expression and silencing (Santoro et al. 2002; Santoro and Grummt 2005). Genes encoding mRNA and rRNA can be found in two different chromatin configurations: an open chromatin structure corresponding to a transcriptionally active state and a more "condensed" configuration representing a silent state. rRNA genes are localized to the nucleolus, a large "transcription factory" (Cook 1999) that is often in the central region of the nucleus. Interestingly, trancriptionally active mRNA genes that occupy Pol II–positive sites are also centrally located and share "transcription factories" (Osborne et al. 2004; Harničarová et al. 2006; Takizawa et al. 2008).

Recently published experiments have shown that specific epigenetic factors, including histone acetylation and methylation, affect the transcription of rRNA genes (Santoro et al. 2002; Santoro and Grummt 2005). This suggests that rDNA transcription is a result of cooperation between DNA methylation, histone modification, and chromatin remodeling factors (McStay and Grummt 2008). This review will summarize current knowledge about the epigenetic regulation of rDNA genes in comparison with the epigenetic and structural regulation of mRNA species.

General Structure of Nucleoli

Mammalian nuclei mainly contain one nucleolus, with tumor cells tending to contain multiple nucleoli. The nucleolus is a non-membrane-bound nuclear compartment and is a relatively dynamic structure, as are some components of the surrounding non-nucleolar chromatin (Phair and Misteli 2000; Misteli 2001; Chen et al. 2005; Olson and Dundr 2005; Shav-Tal et al. 2005). Nucleolar morphology has been correlated with various steps in ribosome biogenesis, and the primary function of the nucleolus is synthesis of ribosomal subunits. NORs, which contain rDNA repeats oriented in a telomere-to-centromere direction, are responsible for formation of nucleoli (Worton et al.1988; Gonzalez and Sylvester 1997). NORs consist of canonical and non-canonical repeats (Caburet et al. 2005), some of which are palindromic. In human chromosomes, rDNA repeats map to short arms of acrocentric chromosomes. However, not all NOR-containing chromosomes associate with nucleoli, and chromosomes without NORs can also surround nucleoli. In some cases, NOR chromosomes that are not associated with nucleoli extend their rDNA-chromatin fibers to the nucleolus, connecting the extranucleolar chromosomal territory with nucleoli (Kalmárová et al. 2008). Some transcriptionally active non-ribosomal genes are located on large chromatin loops (Volpi et al. 2000; Wiblin et al. 2005),

and some mRNA genes associate with the nucleolus (Shimizu et al. 2007; Bártová et al. 2008a; Royo et al. 2009). Thus, in addition to the transcriptional activity of NOR-bearing chromosomes, the transcription and splicing of non-ribosomal genes can be closely linked to the nucleolar compartment.

Many experiments have shown that nucleolar integrity is preferentially maintained by transcription of rRNA genes. The nucleolus contains several important compartments: different domains are responsible for the production of preribosomal particles, the transcription of rRNA genes, the processing of primary transcripts into mature 18S, 5.8S, and 28S rRNA, the addition of proteins to nascent preribosomes, and the incorporation of 5S rRNA, which is synthesized in the nucleoplasm by RNA Pol III (Figure 1A). Nucleoli contain many subdomains, such as the fibrillar center (FC), the dense fibrillar component (DFC), and the granular component (GC), that are visible by transmission electron microscopy after staining. Transcription of ribosomal genes, which is mediated by RNA Pol I, likely occurs at the border between the FC and DFC (González-Melendi et al. 2001; Raška 2003). However, some experiments indicate that rDNA transcription occurs in the FC (e.g. Granboulan and Granboulan 1965; Scheer and Benavente 1990; Raška et al. 1995; Cmarko et al. 2000; Thiry et al. 2000). During the next step of rRNA processing, the pre-rRNA accumulates in the DFC, where posttranscriptional events take place. Maturation of pre-rRNA in the DFC leads to formation of 18S, 5.8S, and 28S rRNA (Figure 1A). Granules in the GC are considered preribosomal particles in various stages of maturation (Shaw and Jordan 1995; Raška et al. 2006). Synthesis of ribosomal subunits takes place in the GC, and nearly mature ribosomal subunits leave the nucleolus and can be observed in the cytoplasm (Granboulan and Granboulan 1965; Olson and Dundr 2005). Nucleolar export likely occurs by simple diffusion, as has been documented for 60S subunits (Politz et al. 2003). Actin and myosin I have been detected within nucleoli, suggesting that motor proteins may be involved in the movement of preribosomal ribonucleoprotein (RNP) complexes through the nucleolus. However, there is no direct evidence that actin and myosin are responsible for the movement of nucleolar pre-rRNP complexes (Philimonenko et al. 2004; Miralles and Visa 2006).

The nucleolus contains many other key proteins. For example, fibrillarin is a small nuclear RNP component and plays an important role in processing of pre-rRNA. Moreover, fibrillarin associates with the U3, U8, and U13 small nuclear RNAs and is located in the DFC (Nicol et al. 2000). Similarly, nucleolin is an abundant protein in the nucleolus that interacts with different proteins and RNA sequences. Nucleolin is involved in regulation of chromatin structure, rDNA transcription,

Figure 1 Structure and organization of nucleoli and nucleolar organizer regions (NORs). (**A**) Illustration of nucleolar structure (adapted from Boisvert et al. 2007), showing the fibrillar center (FC), dense fibrillar component (DFC), and granular component (GC). Transcription of ribosomal genes likely proceeds at the border between the FC and DFC. Pre-rRNA is processed and modified by small nucleolar RNPs (snoRNPs) in the DFC. The 5.8S and 28S rRNA associate with 5S RNA in the GC, involving 18S rRNA. Finally, 40S and 60S ribosome subunits in the nucleoplasm are transported to the cytoplasm, where ribosomes with integrated mRNA are assembled. With permission, Boisvert et al. Nat Rev Mol Cell Biol 8:574–585, 2007. (**B**) Animation of the arrangement of mouse centromeric clusters known as chromocenters (blue) around nucleoli, visualized by an antibody against fibrillarin (red). In the normal (SUV39h wild type) mouse immortalized fibroblasts, nucleoli are larger than in cells with abrogated SUV39h HMT function [SUV39h double null (dn)]. Immortalization likely increases the number of nucleoli. These data show the influence of changes in the histone code on the morphology of nucleoli.
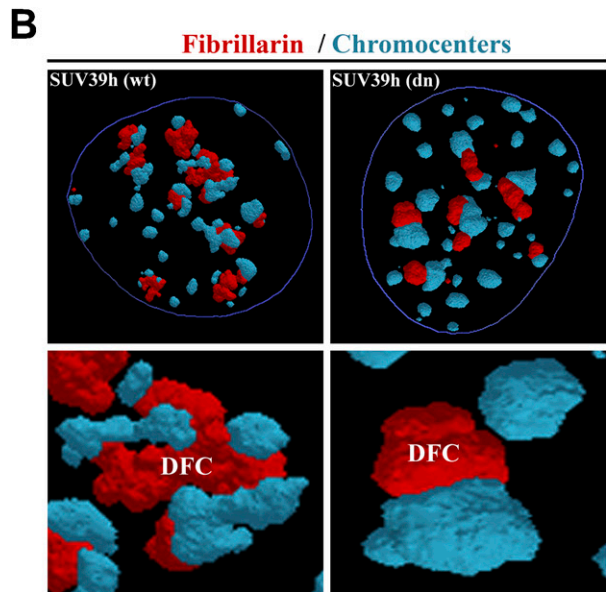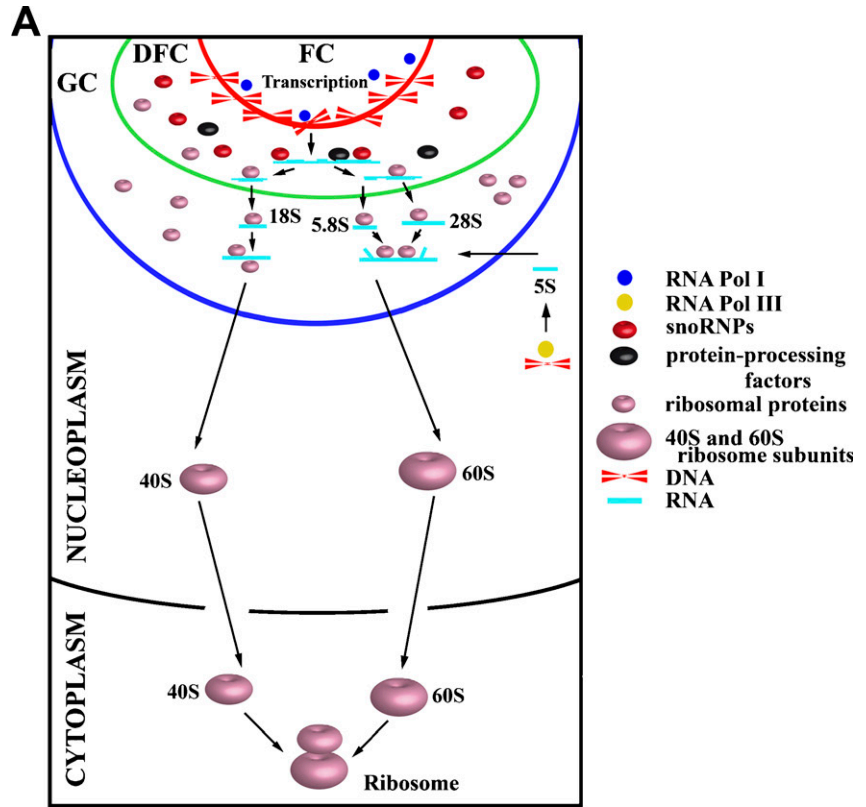
rRNA maturation, ribosome assembly, and nucleocytoplasmic transport (Ginisty et al. 1999). Nucleolin is responsible for chromatin condensation and decondensation and has enhanced affinity to histone 1 (H1) (Erard et al. 1988) in linker regions. These experiments clearly document a close relationship between histone signature and nucleolar function.

Higher-order Chromatin Structure of mRNA and rRNA Genes

Functional significance of nuclear arrangement of mRNA genes is still matter of discussion. Fluorescence in situ hybridization (FISH) was a breakthrough technique in the chromatin architecture field, since it enabled visualization of the spatial nuclear arrangement

of chromosome territories (CTs) and their subdomains (Cremer et al. 1988; Pinkel et al. 1988). Similarly, immunofluorescence combined with high-resolution confocal microscopy permits examination of the functional and structural characteristics of chromatin-related proteins and bodies, including Cajal, promyelocytic leukemia bodies, nuclear speckles containing splicing factors, and others (Zirbel et al. 1993; Clemson et al. 1996; Bridger et al. 1998). There are several general principles governing the nuclear and territorial organization of mRNA genes (Scheuermann et al. 2004). For example, transcriptionally active genes are positioned on the periphery of their CTs, while an anonymous DNA fragment was observed in more interior parts of CTs (Kurz et al. 1996). Moreover, in cells with decondensed chromatin, such as pluripotent embryonic stem cells (ESCs), active genes are positioned on chromatin loops extended away from their CTs (Volpi et al. 2000; Mahy et al. 2002, Williams et al. 2002; Wiblin et al. 2005, Bártová et al. 2008c). Some of these genes share the same "transcription factory" or site of nascent RNA synthesis, even though they are from different territories (Osborne et al. 2004). Extreme chromatin loops are especially prevalent for genes in clusters. For example, the *Oct3/4* genes map to the major histocompatibility gene cluster, and when they are active, they are positioned on chromatin loops in pluripotent human ESCs (hESCs) (Wiblin et al. 2005; Bártová et al. 2008c). However, the *c-myc* gene, mapped in close proximity to the telomere of human chromosome 8, is associated with the periphery of the chromosome territory in pluripotent and differentiated hESCs (Bártová et al. 2008c). Similarly, the *Nanog* gene, which is responsible for hESC pluripotency, is not observed in loops when transcriptionally active; rather, it is positioned in the very interior of the nucleus. Thus, individual genes show distinct structural characteristics that are likely to be influenced by gene density in the surrounding chromatin (Küpper et al. 2007). Generally, GC-rich domains have often been observed closer to the nuclear center, while gene-poor regions occupied the nuclear periphery (Saccone et al. 2002; Goetze et al. 2007). For instance, gene-rich chromosome 19 is positioned closer to the nuclear interior than gene-poor human chromosome 18. The nuclear position of the sequences of these two chromosomes was found to be conserved in several cell types and during evolution (Tanabe et al. 2002; Neusser et al. 2007). On the other hand, inverted nuclear architecture was observed in rod photoreceptor cells in animals with nocturnal vision. The rods of nocturnal retinas have a unique inverted pattern, such that heterochromatin is located in the nuclear center, while transcription of euchromatin and posttranscription processes are associated with the nuclear periphery (Solovei et al. 2009). This inverted pattern of nuclear architecture is not found in other eukaryotic cells and can be explained by adaptation of animals to nocturnal vision.

The arrangement of ~400 copies of the 43-kb human rRNA genes is in some aspects very similar to the nuclear architecture of mRNA genes. Nucleoli are positioned in the interior of the nucleus; thus, highly transcriptionally active rDNA, which accounts for ~50% of nascent RNA synthesis (Bernstein and Allis 2005; Russell and Zomerdijk 2005), occupies the nuclear center. Moreover, ribosomal transcripts are arranged in a formation known as a "Christmas tree" (Miller and Beatty 1969; Mosgoeller et al. 2001), which resembles the positioning of mRNA genes on the loops that extend away from the body of related chromosome territory. In "Christmas tree"-shaped structures, which are visible in electron micrographs, transcriptionally active rRNA genes that carry nascent pre-rRNA are located on the imaginary branches. Arrangement of rRNA genes into "Christmas tree" formation has been observed in many human (Koberna et al. 2002) and plant (Shaw et al. 2002) cells. This structure was first detected by the use of bromouridine labeling, which enabled the visualization of compact "Christmas tree" structures in the plant *Pisum sativum* (González-Melendi et al. 2001). However, there are contradictory data showing exact location of rRNA transcription sites within individual compartments of nuclei (FC, DFC, and GC). Some authors mapped rRNA primary transcripts to the interior of the FC, while other experiments showed that transcription of rRNA genes occurs at the border of the FC and DFC (Granboulan and Granboulan 1965; Raška et al. 1995; Cmarko et al. 2000; Thiry et al. 2000). These discrepancies could be due to the different experimental strategies and approaches used to visualize nucleolar structure. Moreover, cell type specificity may also be a factor.

Efficient transcription of rDNA by Pol I requires the formation of a preinitiation complex (PIC), which includes upstream binding factor (UBF) and selectivity factor 1 (SF1), on the promoter (Prieto and McStay 2005). Surprisingly, recent live imaging experiments showed that only 7–10% of Pol I in the nucleolus participates in transcription. Thus, many Pol I molecules are not engaged in rRNA processes (Dundr et al. 2002a,b). UBF mediates recruitment of Pol I by its direct interaction with this enzyme and/or indirectly via still unknown factors. UBF and other PIC components at the rDNA promoter are responsible for topological changes in the rDNA (Russell and Zomerdijk 2005). Moreover, increased rDNA transcription is related to increased levels of promoter occupancy by the SL1 factor, which directs formation of the PIC complex. Synthesis of rRNA is also influenced by other factors and events, especially by changes in the cell cycle and cell differentiation. For example, the p53 protein,

which is responsible for cell cycle regulation, prevents interaction between UBF and SL1, thus repressing Pol I–mediated transcription (Grummt 2003). Similarly, retinoblastoma (Rb) protein disrupts interactions between UBF and SL1, resulting in decreased Pol I–mediated transcription (Hannan et al. 2000). UBF expression was also changed during differentiation, accompanied by changes in the level of the *c-myc* proto-oncogene (Poortinga et al. 2004). These data indicate that products of proto-oncogenes, such as *c-myc,* and/or the *TP53* and *Rb1* tumor suppressor genes affect rRNA synthesis. Thus, the level and processing of rRNA are likely influenced by mutations in these genes which lead to malignant cell transformation and uncontrolled tumor growth. Therefore, coordinated regulation of the Pol I–mediated transcription machinery, which involves many epigenetic modifications such as pseudouridylation and rRNA methylation, is important for optimal ribosome biogenesis, protein synthesis, cell renewal, growth, differentiation, and cell death.

Many Histone Modifications Are Observed in Both Nucleolar and Non-nucleolar Regions

Eukaryotic chromatin is formed by a complex of core histones (H2A, H2B, H3, and H4) surrounded by 146-bp DNA. These structures are called nucleosomes and are connected to one another by linker DNA that acts as a binding site for H1 (Rice and Allis 2001). Nucleosomes are organized into more coiled structures, leading to the formation of CTs, which are the highest order chromatin structures. CTs are specifically organized within interphase nuclei (Cremer and Cremer 2001; Lanctôt et al. 2007), and mutual contact and/or intermingling between chromosomes territories is currently a topic of interest (Cremer and Cremer 2001; Branco and Pombo 2006; Goetze et al. 2007). According to the model of chromosome nuclear arrangement, with gene-dense territories positioned more interiorly than gene-poor ones (Scheuermann et al. 2004), it is highly probable that histone epigenetic modifications can also dictate nuclear radial arrangement of chromosome subdomains and/or whole territories (Cremer et al. 2004; Zinner et al. 2006; Strašák et al. 2009).

The core N-terminal histone tails are subject to covalent modifications, such as acetylation, methylation, phosphorylation, sumoylation, and/or ubiquitination. The presence of these epigenetic marks is mediated by specific enzymes, including histone acetyltransferases, histone deacetylases (HDACs) that regulate acetylation and/or histone methyltransferases (HMTs), and histone demethylases (Kouzarides 2007; Zhang et al. 2007). These enzymes are responsible for transcriptional activation and/or repression (Hassig et al. 1998; Rice and Allis 2001; Zhang and Reinberg 2001).

Although the existence of histone methylation has been known since 1964 (Murray 1964), only recently has the importance of the histone code in the regulation of nuclear functions been appreciated; indeed, imbalances in this process can lead to human diseases, including cancer (Dialynas et al. 2008; Krejčí et al. 2009).

Histones can be modified at many amino acid positions. There are more then 60 different histone residues at which epigenetic modifications have been identified (Kouzarides 2007). Histone acetylation is one of the best-characterized epigenetic marks and is generally associated with activation of gene expression, while deacetylation causes gene silencing and heterochromatinization (Rice and Allis 2001; Schübeler et al. 2001). However, many other modifications have been detected on histones. Recent studies have shown that methylation at lysines or arginines is an important means of regulating transcription. Moreover, there are three different forms of methylation: mono- and di-methylation at lysines and arginines and tri-methylation at lysines (Kouzarides 2007). Specific histone modifications can be studied by immunohistochemistry, Western blotting, chromatin immunoprecipitation combined with PCR and/or microarray technologies [e.g., chromatin immunoprecipitation (ChIP-on-chip)], and the most sophisticated method, mass spectrometry (Kouzarides 2007).

Global analysis of all histone modifications has shown that some forms of histone methylation, such as H3K4, H3K36, and H3K79, are responsible for transcriptional activation, while H3K9, H3K27, and H4K20 methylation occupies heterochromatin regions and/or transcriptionally silent loci. Moreover, orchestrated activation of Polycomb-group (PcG) protein complexes (PRC1 and PRC2), which act in concert with H3K27me3, leads to inactivation of one female X chromosome as facultative heterochromatin. Some proteins from PcG, especially BMI-1, were also observed within nucleoli (Cmarko D, unpublished data). Interaction of H3K9 methylation with heterochromatin protein 1 (HP1) guarantees the stability of pericentromeric heterochromatin (Lehnertz et al. 2003), which is arranged into chromocenters that surround nucleoli. However, most histone modifications are dynamic, and basic enzymes that mediate and remove specific histone marks were described previously (Kouzarides 2007). For example, acetyltransferases are divided into three families, GNAT, MYST, and CBP/p300, and deacetylation is mediated by class I–III histone deacetylases. SUV39h HMT is responsible for H3K9me3, and G9a HMT is responsible for H3K9me1 and H3K9me2 (Kouzarides 2007). Moreover, recently discovered histone demethylases (Shi et al. 2004; Kouzarides 2007), such as LSD1 (Shi et al. 2004), are responsible for H3K4 demethylation, while methylation at H3K36 can be reversed by the demethylase JHDM1 (Tsukada et al. 2006;

Whetstine et al. 2006). Similarly, murine JMJD2 proteins antagonize H3K9me3 at pericentromeric heterochromatin (Fodor et al. 2006). Thus, JMJD2b could be an important factor that regulates the morphology and composition of nucleoli, which are surrounded by H3K9me3-dense chromocenters.

Histone modifications could also significantly influence higher-order chromatin structure. The histone signature likely affects interactions between histones and DNA, and different modifications can act simultaneously. For example, ubiquitination of H2B is required for H3K4me3 (Kouzarides 2007). LSD1, which is normally responsible for H3K4 demethylation, forms a complex with androgen receptor and causes H3K9 demethylation, thus inducing transcriptional activation (Metzger et al. 2005). These data show that histone epigenetic profiles help to establish euchromatin and heterochromatin domains and thus nuclear architecture. For instance, in early G_1 phase, histone-lysine–methylated sites were scattered throughout the nucleus, whereas during S phase, methylated lysines were located mostly at the nuclear periphery and around nucleoli (Cremer et al. 2004). Similarly, Zinner et al. (2006) and Skalníková et al. (2007) revealed that methylation patterns in interphase nuclei show distinct nuclear layers with a certain degree of overlap, which depends on the type of epigenetic modification. Testing the association of the heterochromatin markers H3K9me3 with H4K27me3 and modifications characterized for active chromatin, including H4K20me1 and H3K4me3, Zinner et al. (2006) showed that specific histone modifications appear in distinct nuclear layers. This observation supports the theory of compartmentalization of interphase chromatin (Cremer and Cremer 2001; Bártová and Kozubek 2006; Lanctôt et al. 2007; Bártová et al. 2008b).

## Epigenetics and Histone Modification in Nucleoli

Similar to the observations in non-nucleolar compartments, transcription of rDNA within the nucleolus (Figure 2A) is regulated by distinct epigenetic marks responsible for both transcriptional silencing and activity (Figures 2B and 2C). The histone signature of acetylation, methylation, and ubiquitination determines the accessibility of chromatin structures for rDNA gene transcription (McStay and Grummt 2008; McKeown and Shaw 2009; Salminen and Kaarniranta 2009). The equilibrium between the epigenetic state and the activity

of chromatin remodeling factors is responsible for the physiological processes that occur within nucleoli. Conversely, non-physiological changes in the nucleolar histone signature can lead to pathological changes with diagnostic significance, especially in oncology (Maggi and Weber 2005). The integrity of the nucleolus is likely maintained by the epigenetic state of rRNA genes, particularly by DNA methyltransferases and histone-modifying enzymes, which act simultaneously with chromatin remodeling complexes involved in rRNA synthesis. These orchestrated processes are responsible for optimal biosynthesis of ribosomes and subsequently for translation. Inhibition of rRNA synthesis leads to rearrangement of the nucleolus, which is accompanied by increased levels of p53 protein, which acts in the nucleolus as a critical stress sensor (Parlato et al. 2008). Moreover, the RNA polymerase inhibitor actinomycin D causes disassembly of nucleoli and loss of mRNA-FISH signals associated with the nucleolar periphery (Royo et al. 2009). Reorganization of nucleoli was also observed after abrogation of DNA methyltransferase 1 (Dnmt1) (Espada et al. 2007) and loss of SUV39h in cells (Figures 2D and 2E). In addition, the functional assembly of the nucleolus is thought to be self-organizing and dynamic (Misteli 2001). The morphology of the nucleolus is directly linked to its functional status and can react to external stimuli (Raška et al. 2004; Louvet et al. 2005; Shav-Tal et al. 2005).

Specific nucleolar functions are also ascribed to the c-Myc protein that accumulates within nucleoli. The c-Myc and Max proteins interact in nucleoli and associate with ribosomal DNA. In addition, c-Myc can activate Pol I transcription, suggesting that c-Myc could play a key role in regulating ribosome biogenesis and subsequently cell growth (Arabi et al. 2005). c-Myc also associates with nucleolar proteins such as Pol I and pontin, a putative mammalian DNA-helicase involved in the c-Myc–dependent regulation of rRNA synthesis (Cvačková et al. 2008). In addition, *c-myc* transcripts occupy nucleoli in various cell types (Bond and Wold 1993; Shimizu et al. 2007; Bártová et al. 2008a). In all cases tested, *c-myc* transcripts were often positioned at the periphery of and/or inside nucleoli, which correlates with the observation that transcription and probably splicing of mRNA genes proceeds in the central part of interphase nuclei (Zink et al. 2004; Harničarová et al. 2006).

The nucleolus (Figure 2A) is characterized by specific epigenetic patterns (Figure 2B). Human cells lacking Dnmt1, but not Dnmt3b, show a loss in DNA

Figure 2 Illustration of epigenetic modifications at rDNA. (**A**) Micrographs of primary mouse embryonic fibroblasts with single, densely stained nucleoli (Nu). (**B**) Detail of nucleolus with known epigenetic modifications to rDNA. (**C**) Scheme of epigenetic regulation of rDNA transcription and silencing. (**D**) Silver-stained NORs (Ag-NORs) in SUV39h wild-type (wt) and (**E**) SUV39h double null (dn) fibroblasts. SUV39h deficiency caused changes in the morphology of Ag-NORs. Bar = 1 μm.
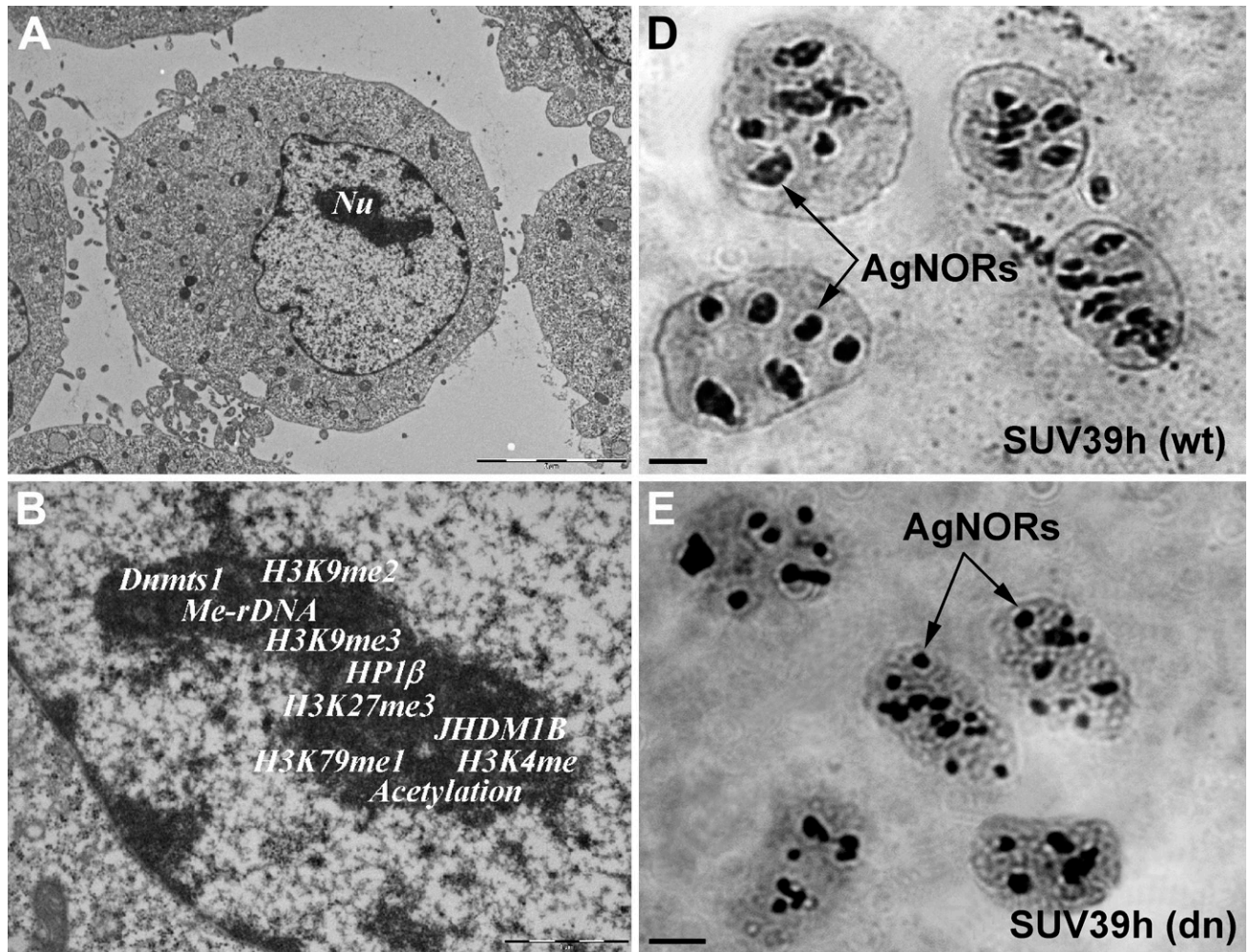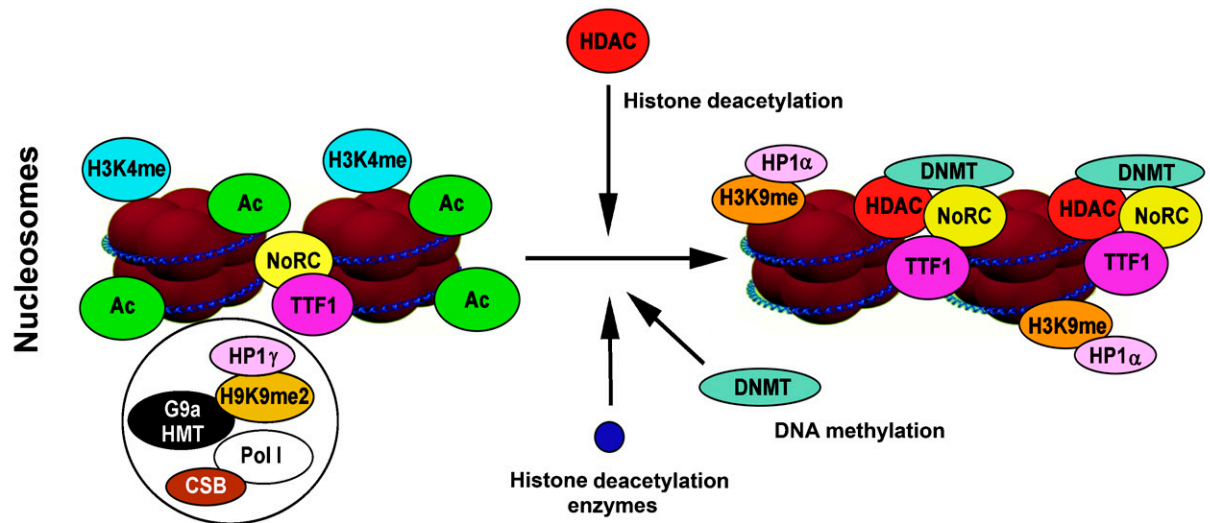
methylation and an increase in the acetylation level of H4K16 in rRNA genes (Espada et al. 2007). Moreover, the recruitment of SirT1, a NAD$^+$-dependent histone deacetylase with a preference for H4K16, to rRNA genes is abrogated in Dnmt1-deficient cells. These events are accompanied by structural disorganization of nucleolus and dispersal of nucleolar proteins, such as fibrillarin (Figure 1B) and pKi-67 [an interphase pattern was published by Harničarová et al. (2006); see also Krejčí et al. (2008)], within the nucleus of the cells carrying mutations. Thus, epigenetic events and factors such as Dnmt1 are likely responsible for the maintenance of stable nucleolar structure and function.

Recent work has also demonstrated that the histone demethylase JHDM1B is located in the nucleolus, which suggests a possible functional role of this enzyme in this compartment. JHDM1B appears to induce H3K4 demethylation and cause transcriptional repression of rDNA genes. However, it is not clear how JHDM1B is targeted to rDNA or how it relates to silencing by the nucleolar remodeling complex (NoRC), which is responsible for nucleosome sliding in an ATP- and H4 tail-dependent manner (Strohner et al. 2001; Frescas et al. 2007; McStay and Grummt 2008). The NoRC is composed of two subunits, the ATPase SNF2h and the 205-kDa TTF-I-interacting protein 5 protein (TIP5) (McStay and Grummt 2008). This complex can interact with Dnmt1, Dnmt3, and the Sin3 correpressor complex containing histone deacetylases HDAC1 and HDAC2 but not SIRT1 (Santoro et al. 2002; Zhou et al. 2002; McStay and Grummt 2008). This complex is subsequently responsible for acetylation and methylation of H3K9, H4K20, and H3K27. This epigenetic mark triggers heterochromatinization, which stabilizes the structure of nucleoli (Salminen and Kaarniranta 2009). Thus, NoRC is an important epigenetic repressor that silences rDNA repeats via DNA methylation and histone deacetylation (Figure 2C). Moreover, overexpression of TIP5, which is required for localization of NoRC to nucleoli in human and mouse cells, results in rDNA silencing (Santoro et al. 2002). Interestingly, TIP5-mediated transcriptional repression of rDNA genes is lost after treatment of cells by Dnmt and HDAC inhibitors, such as 5-azacytidine and trichostatin A (TSA). This

suggests that NoRC may repress rDNA transcription in parallel with inducing DNA methylation and histone deacetylation. DNA methylation induced by the NoRC complex is important for recruitment of UBF to the promoter of rDNA genes and inhibits the activation of Pol I and thus the transcription of rDNA genes.

The balance between activity and silencing of rDNA genes is likely regulated not only by TIP5 and TTF-1, but also by Cockayne syndrome B protein (CSB), which is also involved in transcription of ribosomal genes. CSB, together with SNF-like ATPase and G9a HMT, are responsible for H3K9 dimethylation, which plays an important role in rDNA gene activation (Yuan et al. 2007) (Figure 2C). Thus, H3K9 methylation, which binds heterochromatin protein 1γ (HP1γ), is a surprisingly important epigenetic signature for transcriptionally active rDNA repeats (Yuan et al. 2007) (Figure 2C). Simultaneous activation and silencing of a given gene is not observed only in rDNA genes; monoallelic expression occurs with several mRNA species, including the *c-myc* oncogene and the *CCND1* gene (Levsky et al. 2002). The transcriptionally active allele is positioned more interiorly in the nucleus than the non-active allele (Harničarová et al. 2006; Takizawa et al. 2008). This suggests the simultaneous existence of distinct epigenetic profiles in active and silent genomic regions.

Different epigenetic patterns were also observed after the cell treatment by inhibitors of epigenetic processes such as 5-azacytidine and TSA. We found decreased levels of H3K9me2 in nucleoli after 5-azacytidine treatment (Figure 3A1) and increased levels of H3K4me2 in nucleoli after TSA cell stimulation (Figure 3B). The centromeres of acrocentric chromosomes are known to border the nucleoli. We found dense H3K9me2 at the centromeres of two human acrocentric chromosomes, 14 and 22 (Figure 3A2), while H3K4me2 and H3K9 acetylation was reduced in these centromeric regions (Figure 3C). Similarly, H3K9 acetylation was absent from the centromeres of human submetacentric chromosomes 9 and 10, which frequently occupied the border of nucleoli (Figure 3C3). Surprisingly, even centromere of the human Y chromosome was associated with the nucleolus (Figure 3C2). Thus, close relationships exist between chromatin structure, histone modifications, and arrangement of nucleoli, suggesting that

Figure 3 An example of selected histone modifications and how HDAC and Dnmts inhibitors change their nucleolar compartment localization. (**A1**) H3K9me2 in control and 5-azacytidine–treated human small-cell-lung carcinoma A549 cells. (**A2**) H3K9me2 in centromeric regions of human acrocentric chromosomes 14 and 22 (adapted from Skalníková et al. 2007). (**B**) H3K4me2 in control and TSA-treated A549 cells. TSA caused an increase in H3K4me2 in the nucleolar compartment (white frame). (**C1**) Absence of H3K4me2 at centromeres 14 and 22 (red spots). (**C2**) Association of centromere of human Y chromosome (red spot) with nucleoli. (**C3**) Absence of H3K9 acetylation at centromeres of human chromosomes (HSA) 9 (red) and 10 (green) in control and TSA-treated A549 cells. Both chromosomes associated with nucleoli even though they are not NOR related. Bar = 1 μm. With permission, Skalníková et al. Physiol Res 56:797–806, 2007.
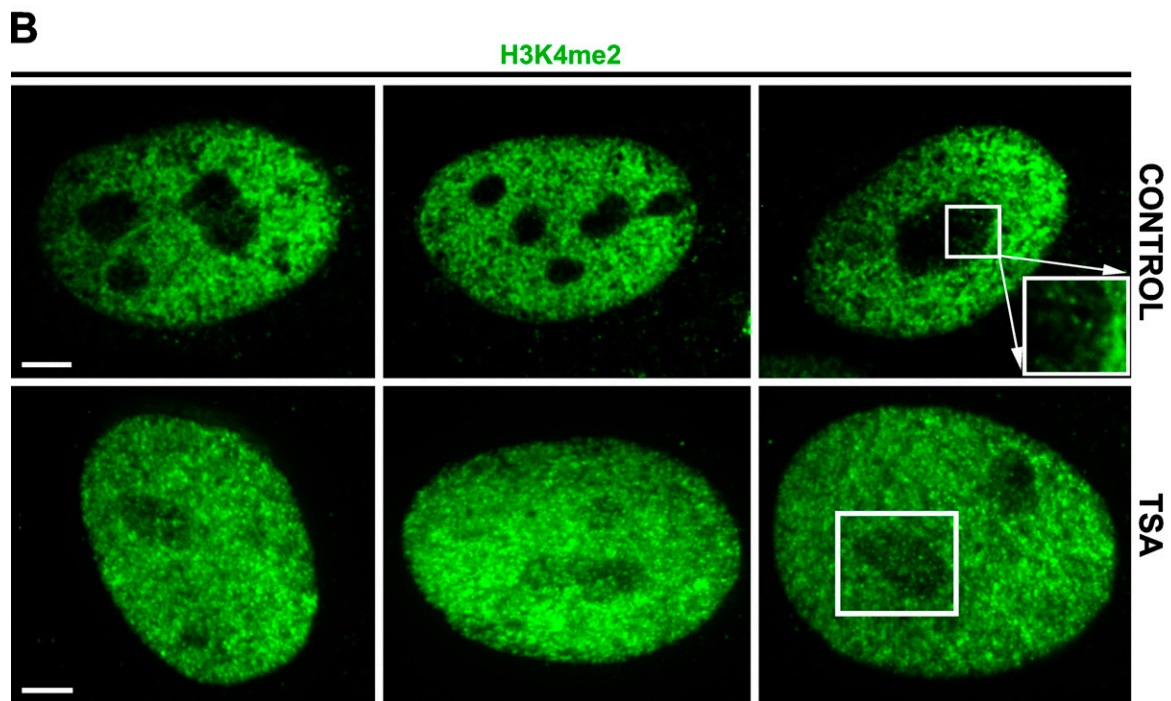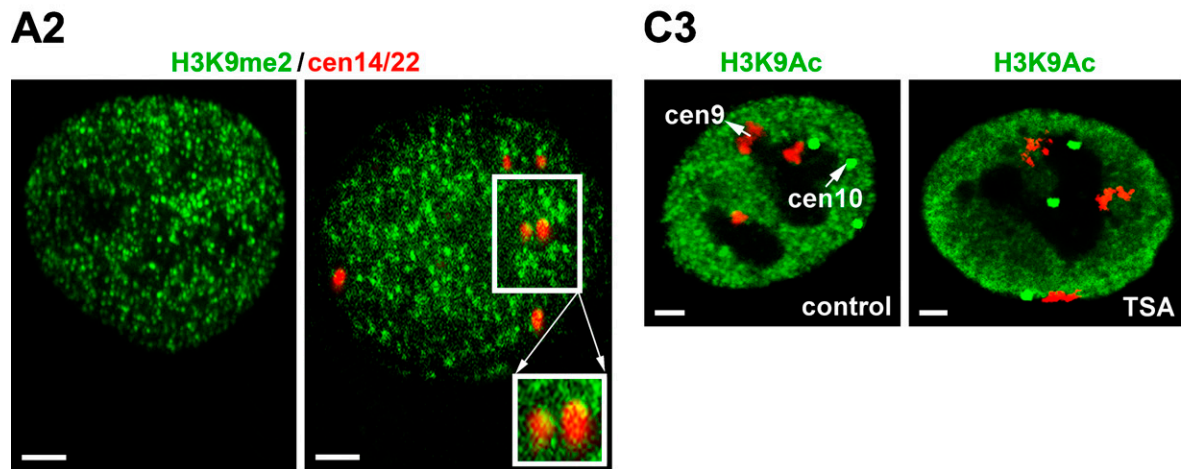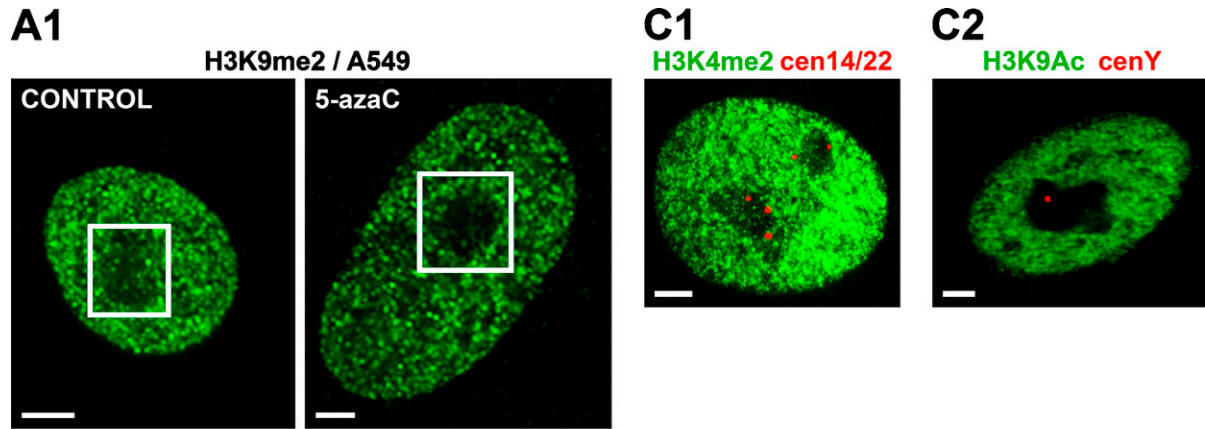
the nucleolus contains many epigenetic factors that are responsible for both rDNA gene silencing and transcriptional activity.

Heterochromatin carries epigenetic marks, such as H3K9 methylation, HP1 protein subtypes (HP1α, HP1β, HP1γ), and rDNA methylation that can be also observed within nucleoli (Pikaard 1999; Kovařík et al. 2000, McStay and Grummt 2008). On the other hand, rDNA genes are also characterized by epigenetic marks specific to the more "open" chromatin configuration of transcriptionally active loci. For instance, histone acetylation has been observed in active rDNA genes (Santoro et al. 2002). However, when NoRC-mediated H4 deacetylation occurs, H3K9 dimethylation and de novo DNA methylation is increased. This leads to modifications of heterochromatin at the rRNA gene promoter. TSA treatment, inducing histone hyperacetylation, does not influence NoRC-mediated H3K9 dimethylation (Santoro et al. 2002), which is in agreement with previous findings that histone acetylation and methylation act independently (Maison et al. 2002; Yan et al. 2003). Moreover, the association of NoRC with chromatin requires H4K16 acetylation, which is required for subsequent deacetylation of H4K5, H4K8, and H4K12, indicating that H4K16 acetylation plays a dominant role in NoRC-mediated heterochromatinization (Zhou and Grummt 2005).

H3K9 methylation is known to be a mark of heterochromatin and transcriptionally silent loci. Interestingly, inhibition of H3K9 methylation by mutation of the *Su(var)3-9* gene destabilizes rDNA, leading to the formation of extrachromosomal rDNA circles and nucleolar rearrangement (Peng and Karpen 2007). This suggests that disrupting the molecular mechanisms that establish the epigenetic state of rRNA genes destabilizes the interactions between DNA methyltransferases, histone-modifying enzymes, and chromatin remodeling complexes that regulate transcription of rDNA genes. Moreover, methylation at rDNA promoters is a dynamic and reversible process (Schmitz et al. 2009), as is the arrangement of nucleoli under external stimuli.

Conclusions

The nucleolus is the largest transcription factory and is a key nuclear compartment for biosynthesis of ribosomal subunits. The dynamic nature of nucleoli has been recently demonstrated, with nucleolar composition changing in response to external stimuli. It is highly probable that the stability of nucleoli is guaranteed by the stability of nucleolar proteins, such as modified histones. The histone signature, together with chromatin remodeling factors, plays an important role in the regulation of rRNA gene transcription, thus influencing the stability and function of nucleoli. Disorders in this process lead to pathophysiological states, including cancer.

Literature Cited

Arabi A, Wu S, Ridderstråle K, Bierhoff H, Shiue C, Fatyol K, Fahlén S, et al. (2005) c-Myc associates with ribosomal DNA and activates RNA polymerase I transcription. Nat Cell Biol 7:303–310

Bártová E, Harničarová A, Krejčí J, Strašák L, Kozubek S (2008a) Single-cell c-myc gene expression in relationship to nuclear domains. Chromosome Res 16:325–343

Bártová E, Kozubek S (2006) Nuclear architecture in the light of gene expression and cell differentiation studies. Biol Cell 98:323–336

Bártová E, Krejčí J, Harničarová A, Galiová G, Kozubek S (2008b) Histone modifications and nuclear architecture: a review. J Histochem Cytochem 56:711–721

Bártová E, Krejčí J, Harničarová A, Kozubek S (2008c) Differentiation of human embryonic stem cells induces condensation of chromosome territories and formation of heterochromatin protein 1 foci. Differentiation 76:24–32

Bernstein E, Allis CD (2005) RNA meets chromatin. Genes Dev 19:1635–1655

Boisvert FM, van Koningsbruggen S, Navascués J, Lamond AI (2007) The multifunctional nucleolus. Nat Rev Mol Cell Biol 8:574–585

Bond VC, Wold B (1993) Nucleolar localization of myc transcripts. Mol Cell Biol 13:3221–3230

Branco MR, Pombo A (2006) Intermingling of chromosome territories in interphase suggests role in translocations and transcription-dependent associations. PLoS Biol 4:e138

Bridger JM, Herrmann H, Münkel C, Lichter P (1998) Identification of an interchromosomal compartment by polymerization of nuclear-targeted vimentin. J Cell Sci 111:1241–1253

Caburet S, Conti C, Schurra C, Lebofsky R, Edelstein SJ, Bensimon A (2005) Human ribosomal RNA gene arrays display a broad range of palindromic structures. Genome Res 15:1079–1085

Chen D, Dundr M, Wang C, Leung A, Lamond A, Misteli T, Huang S (2005) Condensed mitotic chromatin is accessible to transcription factors and chromatin structural proteins. J Cell Biol 168:41–54

Clemson CM, McNeil JA, Willard HF, Lawrence JB (1996) XIST RNA paints the inactive X chromosome at interphase: evidence for a novel RNA involved in nuclear/chromosome structure. J Cell Biol 132:259–275

Cmarko D, Verschure PJ, Rothblum LI, Hernandez-Verdun D, Amalric F, van Driel R, Fakan S (2000) Ultrastructural analysis of nucleolar transcription in cells microinjected with 5-bromo-UTP. Histochem Cell Biol 113:181–187

Cook PR (1999) The organization of replication and transcription. Science 284:1790–1795

Cremer M, Zinner R, Stein S, Albiez H, Wagler B, Cremer C, Cremer T (2004) Three dimensional analysis of histone methylation patterns in normal and tumor cell nuclei. Eur J Histochem 48:15–28

Cremer T, Cremer C (2001) Chromosome territories, nuclear architecture and gene regulation in mammalian cells. Nat Rev Genet 2:292–301

Cremer T, Lichter P, Borden J, Ward DC, Manuelidis L (1988) Detection of chromosome aberrations in metaphase and interphase tumor cells by in situ hybridization using chromosome-specific library probes. Hum Genet 80:235–246

Cvačková Z, Albring KF, Koberna K, Ligasová A, Huber O, Raška I, Staněk D (2008) Pontin is localized in nucleolar fibrillar centers. Chromosoma 117:487–497

Dev VG, Tantravahi R, Miller DA, Miller OJ (1977) Nucleolus organizers in Mus musculus subspecies and in the RAG mouse cell line. Genetics 86:389–398

Dialynas GK, Vitalini MW, Wallrath LL (2008) Linking heterochromatin protein 1 (HP1) to cancer progression. Mutat Res 647:13–20

Dimario PJ (2004) Cell and molecular biology of nucleolar assembly and disassembly. Int Rev Cytol 239:99–178

Dundr M, Hoffmann-Rohrer U, Hu Q, Grummt I, Rothblum LI, Phair RD, Misteli T (2002a) A kinetic framework for a mammalian RNA polymerase in vivo. Science 298:1623–1626

Dundr M, McNally JG, Cohen J, Misteli T (2002b) Quantitation of GFP-fusion proteins in single living cells. J Struct Biol 140:92–99

Erard MS, Belenguer P, Caizergues-Ferrer M, Pantaloni A, Amalric F (1988) A major nucleolar protein, nucleolin, induces chromatin decondensation by binding to histone H1. Eur J Biochem 175:525–530

Espada J, Ballestar E, Santoro R, Fraga MF, Villar-Garea A, Németh A, Lopez-Serra L, et al. (2007) Epigenetic disruption of ribosomal RNA genes and nucleolar architecture in DNA methyltransferase 1 (Dnmt1) deficient cells. Nucleic Acids Res 35:2191–2198

Fodor BD, Kubicek S, Yonezawa M, O'Sullivan RJ, Sengupta R, Perez-Burgos L, Opravil S, et al. (2006) Jmjd2b antagonizes H3K9 trimethylation at pericentric heterochromatin in mammalian cells. Genes Dev 20:1557–1562

Frescas D, Guardavaccaro D, Bassermann F, Koyama-Nasu R, Pagano M (2007) JHDM1B/FBXL10 is a nucleolar protein that represses transcription of ribosomal RNA genes. Nature 450:309–313

Ginisty H, Sicard H, Roger B, Bouvet P (1999) Structure and functions of nucleolin. J Cell Sci 112:761–772

Goetze S, Mateos-Langerak J, Gierman HJ, de Leeuw W, Giromus O, Indemans MH, Koster J, et al. (2007) The three-dimensional structure of human interphase chromosomes is related to the transcriptome map. Mol Cell Biol 27:4475–4487

Gonzalez IL, Sylvester JE (1997) Beyond ribosomal DNA: on towards the telomere. Chromosoma 105:431–437

González-Melendi P, Wells B, Beven AF, Shaw PJ (2001) Single ribosomal transcription units are linear, compacted Christmas trees in plant nucleoli. Plant J 27:223–233

Granboulan N, Granboulan P (1965) Ultrastructure cytochemistry of the nucleolus. II. Study of the sites of RNA synthesis in the nucleolus and the nucleus. Exp Cell Res 38:604–619

Grummt I (2003) Life on a planet of its own: regulation of RNA polymerase I transcription in the nucleolus. Genes Dev 17:1691–1702

Hannan KM, Hannan RD, Smith SD, Jefferson LS, Lun M, Rothblum LI (2000) Rb and p130 regulate RNA polymerase I transcription: Rb disrupts the interaction between UBF and SL-1. Oncogene 19:4988–4999

Harničarová A, Kozubek S, Pacherník J, Krejčí J, Bártová E (2006) Distinct nuclear arrangement of active and inactive c-myc genes in control and differentiated colon carcinoma cells. Exp Cell Res 312:4019–4035

Hassig CA, Tong JK, Fleischer TC, Owa T, Grable PG, Ayer DE, Schreiber SL (1998) A role for histone deacetylase activity in HDAC1-mediated transcriptional repression. Proc Natl Acad Sci USA 95:3519–3524

Henderson AS, Warburton D, Atwood KC (1972) Location of ribosomal DNA in the human chromosome complement. Proc Natl Acad Sci USA 69:3394–3398

Kalmárová M, Smirnov E, Kováčik L, Popov A, Raška I (2008) Positioning of the NOR-bearing chromosomes in relation to nucleoli in daughter cells after mitosis. Physiol Res 57:421–425

Koberna K, Malínský J, Pliss A, Masata M, Vecerova J, Fialová M, Bednár J, et al. (2002) Ribosomal genes in focus: new transcripts label the dense fibrillar components and form clusters indicative of "Christmas trees" in situ. J Cell Biol 157:743–748

Kouzarides T (2007) Chromatin modifications and their function. Cell 128:693–705

Kovařík A, Koukalová B, Lim KY, Matyášek R, Lichtenstein CP, Leitch AR, Bezděk M (2000) Comparative analysis of DNA methylation in tobacco heterochromatic sequences. Chromosome Res 8:527–541

Krejčí J, Harničarová A, Kůrová J, Uhlířová R, Kozubek S, Legartová S, Hájek R, et al. (2008) Nuclear organization of PML bodies in leukaemic and multiple myeloma cells. Leuk Res 32:1866–1877

Krejčí J, Harničarová A, Streitová D, Hájek R, Pour L, Kozubek S, Bártová E (2009) Epigenetics of multiple myeloma after treatment with cytostatics and gamma radiation. Leuk Res 33:1490–1498

Küpper K, Kölbl A, Biener D, Dittrich S, von Hase J, Thormeyer T, Fiegler H, et al. (2007) Radial chromatin positioning is shaped by local gene density, not by gene expression. Chromosoma 116:285–306

Kurz A, Lampel S, Nickolenko JE, Bradl J, Benner A, Zirbel RM, Cremer T, et al. (1996) Active and inactive genes localize preferentially in the periphery of chromosome territories. J Cell Biol 135:1195–1205

Lanctôt C, Cheutin T, Cremer M, Cavalli G, Cremer T (2007) Dynamic genome architecture in the nuclear space: regulation of gene expression in three dimensions. Nat Rev Genet 8:104–115

Lehnertz B, Ueda Y, Derijck AA, Braunschweig U, Perez-Burgos L, Kubicek S, Chen T, et al. (2003) SUV39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. Curr Biol 13:1192–1200

Leung AK, Gerlich D, Miller G, Lyon C, Lam YW, Lleres D, Daigle N, et al. (2004) Quantitative kinetic analysis of nucleolar breakdown and reassembly during mitosis in live human cells. J Cell Biol 166:787–800

Levsky JM, Shenoy SM, Pezo RC, Singer RH (2002) Single-cell gene expression profiling. Science 297:836–840

Louvet E, Junéra HR, Le Panse S, Hernandez-Verdun D (2005) Dynamics and compartmentation of the nucleolar processing machinery. Exp Cell Res 304:457–470

Maggi LB Jr, Weber JD (2005) Nucleolar adaptation in human cancer. Cancer Invest 23:599–608

Mahy NL, Perry PE, Bickmore WA (2002) Gene density and transcription influence the localization of chromatin outside of chromosome territories detectable by FISH. J Cell Biol 159:753–763

Maison C, Bailly D, Peters AH, Quivy JP, Roche D, Taddei A, Lachner M, et al. (2002) Higher-order structure in pericentric heterochromatin involves a distinct pattern of histone modification and an RNA component. Nat Genet 30:329–334

McKeown PC, Shaw PJ (2009) Chromatin: linking structure and function in the nucleolus. Chromosoma 118:11–23

McStay B, Grummt I (2008) The epigenetics of rRNA genes: from molecular to chromosome biology. Annu Rev Cell Dev Biol 24:131–157

Metzger E, Wissmann M, Yin N, Müller JM, Schneider R, Peters AH, Günther T, et al. (2005) LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. Nature 437:436–439

Miller OL Jr, Beatty BR (1969) Visualization of nucleolar genes. Science 164:955–957

Miralles F, Visa N (2006) Actin in transcription and transcription regulation. Curr Opin Cell Biol 18:261–266

Misteli T (2001) The concept of self-organization in cellular architecture. J Cell Biol 155:181–185

Mosgoeller W, Schöfer C, Steiner M, Sylvester JE, Hozák P (2001) Arrangement of ribosomal genes in nucleolar domains revealed by detection of "Christmas tree" components. Histochem Cell Biol 116:495–505

Murray K (1964) The occurrence of epsilon-N-methyl lysine in histones. Biochemistry 3:10–15

Neusser M, Schubel V, Koch A, Cremer T, Müller S (2007) Evolutionarily conserved, cell type and species-specific higher order chromatin arrangements in interphase nuclei of primates. Chromosoma 116:307–320

Nicol SM, Causevic M, Prescott AR, Fuller-Pace FV (2000) The nuclear DEAD box RNA helicase p68 interacts with the nucleolar

protein fibrillarin and colocalizes specifically in nascent nucleoli during telophase. Exp Cell Res 257:272–278

Olson MO, Dundr M (2005) The moving parts of the nucleolus. Histochem Cell Biol 123:203–216

Osborne CS, Chakalova L, Brown KE, Carter D, Horton A, Debrand E, Goyenechea B, et al. (2004) Active genes dynamically colocalize to shared sites of ongoing transcription. Nat Genet 36:1065–1071

Parlato R, Kreiner G, Erdmann G, Rieker C, Stotz S, Savenkova E, Berger S, et al. (2008) Activation of an endogenous suicide response after perturbation of rRNA synthesis leads to neurodegeneration in mice. J Neurosci 28:12759–12764

Peng JC, Karpen GH (2007) H3K9 methylation and RNA interference regulate nucleolar organization and repeated DNA stability. Nat Cell Biol 9:25–35

Phair RD, Misteli T (2000) High mobility of proteins in the mammalian cell nucleus. Nature 404:604–609

Philimonenko VV, Zhao J, Iben S, Dingová H, Kyselá K, Kahle M, Zentgraf H, et al. (2004) Nuclear actin and myosin I are required for RNA polymerase I transcription. Nat Cell Biol 6:1165–1172

Pikaard CS (1999) Nucleolar dominance and silencing of transcription. Trends Plant Sci 4:478–483

Pinkel D, Landegent J, Collins C, Fuscoe J, Segraves R, Lucas J, Gray J (1988) Fluorescence in situ hybridization with human chromosome-specific libraries: detection of trisomy 21 and translocations of chromosome 4. Proc Natl Acad Sci 85:9138–9142

Politz JC, Tuft RA, Pederson T (2003) Diffusion-based transport of nascent ribosomes in the nucleus. Mol Biol Cell 14:4805–4812

Poortinga G, Hannan KM, Snelling H, Walkley CR, Jenkins A, Sharkey K, Wall M, et al. (2004) MAD1 and c-MYC regulate UBF and rDNA transcription during granulocyte differentiation. EMBO J 23:3325–3335

Prieto JL, McStay B (2005) Nucleolar biogenesis: the first small steps. Biochem Soc Trans 33:1441–1443

Raška I (2003) Oldies but goldies: searching for Christmas trees within the nucleolar architecture. Trends Cell Biol 13:517–525

Raška I, Dundr M, Koberna K, Melčák I, Risueno MC, Török I (1995) Does the synthesis of ribosomal RNA take place within nucleolar fibrillar centers or dense fibrillar components? A critical appraisal. J Struct Biol 114:1–22

Raška I, Koberna K, Malínský J, Fidlerová H, Mašata M (2004) The nucleolus and transcription of ribosomal genes. Biol Cell 96:579–594

Raška I, Shaw PJ, Cmarko D (2006) New insights into nucleolar architecture and activity. Int Rev Cytol 255:177–235

Rice JC, Allis CD (2001) Histone methylation versus histone acetylation: new insights into epigenetic regulation. Curr Opin Cell Biol 13:263–273

Royo F, Paz N, Espinosa L, McQueen PG, Vellón L, Parada LA (2009) Spatial link between nucleoli and expression of the Zac1 gene. Chromosoma 118:711–722

Russell J, Zomerdijk JC (2005) RNA-polymerase-I-directed rDNA transcription, life and works. Trends Biochem Sci 30:87–96

Saccone S, Federico C, Bernardi G (2002) Localization of the gene-richest and the gene-poorest isochores in the interphase nuclei of mammals and birds. Gene 300:169–178

Salminen A, Kaarniranta K (2009) SIRT1 regulates the ribosomal DNA locus: epigenetic candles twinkle longevity in the Christmas tree. Biochem Biophys Res Commun 378:6–9

Santoro R, Grummt I (2005) Epigenetic mechanism of rRNA gene silencing: temporal order of NoRC-mediated histone modification, chromatin remodeling, and DNA methylation. Mol Cell Biol 25:2539–2546

Santoro R, Li J, Grummt I (2002) The nucleolar remodeling complex NoRC mediates heterochromatin formation and silencing of ribosomal gene transcription. Nat Genet 32:393–396

Scheer U, Benavente R (1990) Functional and dynamic aspects of the mammalian nucleolus. Bioessays 12:14–21

Scheuermann MO, Tajbakhsh J, Kurz A, Saracoglu K, Eils R, Lichter P (2004) Topology of genes and nontranscribed sequences in human interphase nuclei. Exp Cell Res 301:266–279

Schmitz KM, Schmitt N, Hoffmann-Rohrer U, Schäfer A, Grummt I, Mayer C (2009) TAF12 recruits Gadd45a and the nucleotide excision repair complex to the promoter of rRNA genes leading to active DNA demethylation. Mol Cell 33:344–353

Schübeler D, Groudine M, Bender MA (2001) The murine beta-globin locus control region regulates the rate of transcription but not the hyperacetylation of histones at the active genes. Proc Natl Acad Sci USA 98:11432–11437

Shav-Tal Y, Blechman J, Darzacq X, Montagna C, Dye BT, Patton JG, Singer RH, et al. (2005) Dynamic sorting of nuclear components into distinct nucleolar caps during transcriptional inhibition. Mol Biol Cell 16:2395–2413

Shaw PJ, Abranches R, Paula Santos A, Beven AF, Stoger E, Wegel E, González-Melendi P (2002) The architecture of interphase chromosomes and nucleolar transcription sites in plants. J Struct Biol 140:31–38

Shaw PJ, Jordan EG (1995) The nucleolus. Annu Rev Cell Dev Biol 11:93–121

Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, Casero RA, et al. (2004) Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell 119:941–953

Shimizu N, Kawamoto JK, Utani K (2007) Regulation of c-myc through intranuclear localization of its RNA subspecies. Biochem Biophys Res Commun 359:806–810

Skalníková M, Bártová E, Ulman V, Matula P, Svoboda D, Harnicarová A, Kozubek M, et al. (2007) Distinct patterns of histone methylation and acetylation in human interphase nuclei. Physiol Res 56:797–806

Solovei I, Kreysing M, Lanctôt C, Kösem S, Peichl L, Cremer T, Guck J, et al. (2009) Nuclear architecture of rod photoreceptor cells adapts to vision in mammalian evolution. Cell 137:356–368

Strašák L, Bártová E, Harničarová A, Galiová G, Krejčí J, Kozubek S (2009) H3K9 acetylation and radial chromatin positioning. J Cell Physiol 220:91–101

Strohner R, Nemeth A, Jansa P, Hofmann-Rohrer U, Santoro R, Längst G, Grummt I (2001) NoRC: a novel member of mammalian ISWI-containing chromatin remodeling machines. EMBO J 20:4892–4900

Takizawa T, Gudla PR, Guo L, Lockett S, Misteli T (2008) Allele-specific nuclear positioning of the monoallelically expressed astrocyte marker GFAP. Genes Dev 22:489–498

Tanabe H, Müller S, Neusser M, von Hase J, Calcagno E, Cremer M, Solovei I, et al. (2002) Evolutionary conservation of chromosome territory arrangements in cell nuclei from higher primates. Proc Natl Acad Sci USA 99:4424–4429

Thiry M, Cheutin T, O'Donohue MF, Kaplan H, Ploton D (2000) Dynamics and three-dimensional localization of ribosomal RNA within the nucleolus. RNA 6:1750–1761

Tsukada Y, Fang J, Erdjument-Bromage H, Warren ME, Borchers CH, Tempst P, Zhang Y (2006) Histone demethylation by a family of JmjC domain-containing proteins. Nature 439:811–816

Volpi EV, Chevret E, Jones T, Vatcheva R, Williamson J, Beck S, Campbell RD, et al. (2000) Large-scale chromatin organization of the major histocompatibility complex and other regions of human chromosome 6 and its response to interferon in interphase nuclei. J Cell Sci 113:1565–1576

Whetstine JR, Nottke A, Lan F, Huarte M, Smolikov S, Chen Z, Spooner E, et al. (2006) Reversal of histone lysine trimethylation by the JMJD2 family of histone demethylases. Cell 125:467–481

Wiblin AE, Cui W, Clark AJ, Bickmore WA (2005) Distinctive nuclear organisation of centromeres and regions involved in pluripotency in human embryonic stem cells. J Cell Sci 118:3861–3868

Williams RR, Broad S, Sheer D, Ragoussis J (2002) Subchromosomal positioning of the epidermal differentiation complex (EDC) in keratinocyte and lymphoblast interphase nuclei. Exp Cell Res 272:163–175

Worton RG, Sutherland J, Sylvester JE, Willard HF, Bodrug S, Dubé I, Duff C, et al. (1988) Human ribosomal RNA genes: orientation of the tandem array and conservation of the 5′ end. Science 239:64–68

Yan Q, Cho E, Lockett S, Muegge K (2003) Association of Lsh, a regulator of DNA methylation, with pericentromeric heterochromatin is dependent on intact heterochromatin. Mol Cell Biol 23:8416–8428

Yuan X, Feng W, Imhof A, Grummt I, Zhou Y (2007) Activation of RNA polymerase I transcription by cockayne syndrome group B protein and histone methyltransferase G9a. Mol Cell 27:585–595

Zhang LF, Huynh KD, Lee JT (2007) Perinucleolar targeting of the inactive X during S phase: evidence for a role in the maintenance of silencing. Cell 129:693–706

Zhang Y, Reinberg D (2001) Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails. Genes Dev 15:2343–2360

Zhou Y, Grummt I (2005) The PHD finger/bromodomain of NoRC interacts with acetylated histone H4K16 and is sufficient for rDNA silencing. Curr Biol 15:1434–1438

Zhou Y, Santoro R, Grummt I (2002) The chromatin remodeling complex NoRC targets HDAC1 to the ribosomal gene promoter and represses RNA polymerase I transcription. EMBO J 21:4632–4640

Zink D, Amaral MD, Englmann A, Lang S, Clarke LA, Rudolph C, Alt F, et al. (2004) Transcription-dependent spatial arrangements of CFTR and adjacent genes in human cell nuclei. J Cell Biol 166:815–825

Zinner R, Albiez H, Walter J, Peters AH, Cremer T, Cremer M (2006) Histone lysine methylation patterns in human cell types are arranged in distinct three-dimensional nuclear zones. Histochem Cell Biol 125:3–19

Zirbel RM, Mathieu UR, Kurz A, Cremer T, Lichter P (1993) Evidence for a nuclear compartment of transcription and splicing located at chromosome domain boundaries. Chromosome Res 1:93–106